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FORMULATION AND STATISTICAL OPTIMIZATION OF S-SMEDDS OF NICARDIPINE HYDROCHLORIDE BY USING BBD AND PCA DESIGN

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Keywords:

Nicardipine Hydrochloride, Self-microemulsifying, Box-Behnken design, Principal Component Analysis, Solid-SMEDDS.

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ABSTRACT: The study purpose is to further enhance the rate of dissolution for poorly water-soluble drug Nicardipine Hydrochloride by dissolving NH in surfactant, Co-surfactant, and oil; phase diagram was plotted by using oil (Capmul MM C8 EP), surfactant (Labrasol), and co-surfactant (PEG 400) through which a region called microemulsifying region was obtain. The batches were optimized by holding a 3-factor and 3-level Box–Behnken design for knowing the impact of independent and dependent variables. For the selection of dependent responses, the PCA technique was employed for examining significant variables. The Liq-SMEDDS were characterized for % T, zeta potential, emulsification time, particle size, cloud point, drug content, etc. The optimized batch shows an emulsification time of 32.73 sec and particle size as 97.745 nm. *In-vitro* drug release shows the faster release of drug as 90.37% within 15 min. S-SMEDDS were obtained by using Neusilin as an adsorbent in Optimized formulation. DSC of solid SMEDDS showed no peak indicates that the drug was completely converted into an amorphous form or was present in solubilized form. From SEM, it was revealed that S-SMEDDS appeared as smooth-surfaced, which indicated that the liquid SMEDDS is adsorbed or coated within the pores of Neusilin US2. TEM results showed that globules of all composition formulas were well dispersed and no aggregation of globule was observed.

INTRODUCTION: One of the most convincible drug administration routes in many diseases is the oral route. Almost 50% of new molecule candidates undergo low water-insoluble problem and dissolution rate, also the delivery from the oral route of such molecule is recurrently connected with low bioavailability problem [8].

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As connected to the crucial pressure of solubility & rate of dissolution for bioavailability, different formulation strategy for enriching the solubility and/or rate of dissolution of (API) Active Pharmaceutical Ingredient are investigated, *i.e*., usage of lipid, surfactant, micronization, the formation of salt, cyclodextrins complex, nano-particles, solid dispersions, *etc*.

In current years, a large amount of awareness has been directed on formulations based on lipids to recover the bioavailability by the oral route of low aqueous soluble molecule candidates (drug). Accessibility of the drug for assimilation can be higher by the appearance of the drug solubilisation

within a colloidal dispersion. Lipid-based delivery includes oil solution, solid dispersion, suspension, SMEDDS, emulsion, and liposomes. These days, the most widely used method for the drug is their conversion into SEDDS and particularly in SMEDDS. It is isotropic homogenous mixtures of a lively compound in an amalgamation of synthetic or natural lipids, co-surfactants, and surfactant [14]. These mixtures are anhydrous are mostly termed pre-concentrates. Upon slight average interruption in aqueous phase, For example, In GI lumen content, these mixtures instinctively form API-encapsulated oil/water micro-emulsions with an average diameter of ≤ 200 nm. In comparison to other formulations, SMEDDS are very thermo dynamically stable formulations [4]. SMEDDS packed into soft/hard gelatin capsules or HPMC capsules results in striking commercial feasibility and compliance of the patient. One of the key features is that the quantity of dose can be limited to achieve a maximal 1 g of formulated liquid that can be contained in a soft gelatin capsule [2, 9, 13].

MATERIALS AND METHODOLOGY: Nicardipine Hydrochloride procured from ZIM Laboratories Ltd. (Nagpur, India) as souvenir sample. Capmul MCM C8 EP was procured from Abitech, Labrasol from Gattefose (France). PEG 400 from Astron Chemicals (India) Pvt. Ltd. Neusilin US2 was taken from Gangawal chemical Ltd, Mumbai. Aerosil-200 was taken from Astron Chemicals. Croscarmellose sodium was procured from Seva fine chemicals, Ahmedabad. Magnesium stearate was taken from Loba Chemie, All these listed materials were procured as a gift sample, and Analytical grades solvents and excipients were used.

Solubility Studies: The intention of solubility is to establish the ability of solubilization for API in a different solvent. Solvent illustrating uppermost solubility is to be used for formulating SMEDDS. Nicardipine Hydrochloride solubility was checked in a range of solvent, *viz*. oils (Castor oil, Olive oil, Sunflower oil, Oleic acid, Capmul MCM C8 EP, Labrafil M1944 CS, Capmul MCM EP, Captex 300 EP, and Acrysol K-140), surfactants (Tween 20/60/80, Cremophor RH40/RH188, Labrasol and co-surfactants (PEG 200/400/600 and Capryol PGMC) was stubborn at first. The solubility of Nicardipine Hydrochloride in diverse oils & surfactants was resolute by adding the surplus amount of API in 2 gm of oils taken independently in 10 ml volume vials with stopper and assorted by use of a mixer. The mixture was trembled for 48 hrs at 40 ± 0.5 °C in a shaker and kept aside for 24 h at room temperature (25 °C) to attain equilibrium. Equilibrated samples were further centrifuged for 15 min at 3000 rpm, 1 ml of supernatant diluted with methanol, and filtered through. What mann-35 paper and solubility were further quantified UV [14].

Pseudo Ternary Phase Diagram: By the outcome of solubility studies, Capmul MCM C8 EP (Oil), PEG 400 (Co-Surfactant), and Labrasol (Surfactant) were selected correspondingly. A ternary diagram was constructed. The sequence of self- micro emulsifying system (SMES) was made with unreliable weight ratio for Oil (10–35% w/w), Surfactant (35–85% w/w), and Co- surfactant (0–25% w/w). Formulations were poured into 200 ml of distilled water for their %T in UV. In the ternary diagram Self-emulsification area was pointed using the ratio of weight of constituent that shows 100% T 3, [10.] A Diagram of ternary is shown in **Fig. 1**.

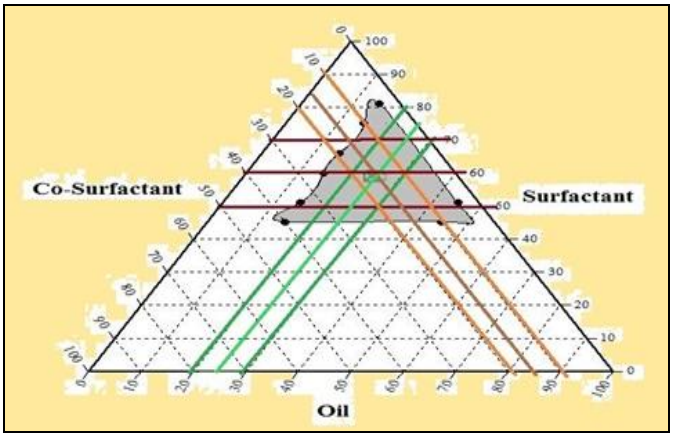


FIG. 1: TERNARY PLOT DIAGRAM OF SURFACTANT (LABRASOL), OIL (CAPMULMM C8 EP) AND CO-SURFACTANT (PEG 400)

Smedds Formulation Preparation: A Box–Behnken (3-factor/level) statistical design by usage of Design Expert was engaged to prepare liq-SMEDDS and was tested for main and interactive effects of independent variables on the formulation. On the basis of trials (preliminary), independent variables (*i.e.*, factors) were fixed as Oil Quantity (X1), Surfactant Quantity (X2), and Co-Surfactant Quantity (X3), while Emulsification time (Y1) and Particle size (Y2) were chosen as the response.

TABLE 1: TRANSLATION OF CODED VALUE IN ACTUAL UNITS

Independent variables	Variable level (parts)		
	(-1) Low	(0) Medium	(+1) High
Oil (X_1)	20	25	30
Surfacatant (X_2)	50	60	70
Co- Surfacatant (X_3)	10	15	20

TABLE 2: EXPERIMENTAL BATCHES FOR SMEDDS USING BOX-BEHNKEN DESIGN

Groups	Quantity of oil (Parts) X_1	Quantity of surfactant (Parts) X_2	Quantity of co-surfactant (Parts) X_3	Emulsification Time (Sec)	Particle Size (nm)
NH1	40	100	30	38.40	102.7
NH2	60	100	30	41.63	108.4
NH3	40	140	30	19.33	92.4
NH4	60	140	30	25.63	98.2
NH5	40	120	20	35.40	100.2
NH6	60	120	20	42.27	109.7
NH7	40	120	40	20.37	92.54
NH8	60	120	40	27.70	100.4
NH9	50	100	20	56.40	112.4
NH10	50	140	20	43.57	107.22
NH11	50	100	40	49.67	106.32
NH12	50	140	40	28.33	94.6
NH13	50	120	30	32.47	98.72

The dependent/ independent variables are shown in **Table 2**. This design is appropriate to explore quadratic response by constructing second-order polynomial models. This cubic design forms a multidimensional cube, and a center point replicate is known by a group of points at each edge of the midpoint. The standard result by changing 1 factor at a time from the lowest rate to highest rate was denoted by the main effects, mainly X1, X2 & X3. When two factors are modified, concurrently, the interaction terms X1X2, X1X3 & X2X3 also change in a timely. Further, the polynomial terms mainly X12, X22 & X32 can be added for non-linearity examination of the model. Initially, the oil phase and surfactant were precisely weighed in screw capped glass vials and were warmed in a water batch at 37 ºC. Further required quantity of desired co-surfactant already been loaded with Nicardipine Hydrochloride was added to vials stirred tenderly for 20 min resulting drug concentration of 20 mg/unit dose. Additionally, the components of optimized batch (*i.e.*, checkpoint) were obtained by construction of an overlay plot.

Characterization of Nicardipine Hydrochloride SMEDDs:

Drug Content: Nicardipine Hydrochloride equivalent to 20 mg in liquid self microemulsion was mixed in 5 ml of methanol. The resultant solution filtered all the way through filter paper and analyzed by UV at 236 nm after further required dilutions by methanol.

Phase Separation Study: In almost 5 ml of distilled water, 1 ml of Nicardipine Hydrochloride SMEDDS was added into a test tube at 25 ºC and vortexed for approx. 1 min. Further, the resulting mixture was stored for a period of 2 h at 25 ºC and observed for any separation (phase), if any.

Transmittance Test: Liquid sample of the self-microemulsion was analyzed through UV-visible spectrophotometer 6 at 236 nm in triplicate for measuring its % transmittance. The motto of the test was to check its stability [16].

Dilution Study: After oral administration, to mimic physiological dilution and to know the dilution effect on SMEDDS pre-concentrates, dilution studies were carried out. At various dilutions in 0.1 N HCl (*i.e.*, 50, 100, 500, and 1000 times), dilution studies of optimized batches of Nicardipine Hydrochloride SMEDDS were carried out. Every diluted sample of SMEDDS was stored for 24 h to observe any signs of phase separation on dilution.

Self-Emulsification Time: By using USP Type II (Paddle type) emulsification time of Nicardipine HCl SMEDDS was determined. The SMEDDS of Nicardipine Hydrochloride poured into 300 ml of

purified water at 37 °C at 50 rpm, and the time required to form clear dispersion was noted as emulsification time [11].

Cloud Point Determination: Nicardipine HCl SMEDDS consists of non-ionic surfactants in order to formation a stable microemulsion the cloud point should be higher than 37 °C to avoid any separation of phase in GI tract. SMEDDS formulation of Nicardipine Hydrochloride mixed in a ratio of 1: 250 in water and kept in water bath gradually increasing temperature.

Zeta-Potential Determination: By use of Zetasizer, Zeta-potential was examined, 1 ml formulation (SMEDDS) was dissolved in 250 ml distilled water with continuous stirring in a glass beaker [11].

Globule Size Determination: Each globule size of microemulsion was measured as a crucial factor in self-emulsification formation, as it examines the rate and extent of release of the drug. Almost 20 mg of every SMEDDS formulation was mixed with 250 ml of water in a glass beaker with constant stirring and examined through zetasizer [12].

***In-vitro* Drug Release:** Study of In-vitro release pattern of SMEDDS of Nicardipine Hydrochloride was performed using USP apparatus I at 37 ± 0.5 ○C on a rotating speed at 100 rpm in 0.1 N HCl. During the profiling, aliquots of 5 ml were pipetted out at (*i.e*. 5, 10, 15, 20, 25, and 30 min) predetermined time intervals from the medium and replaced with fresh media. The quantity of Nicardipine Hydrochloride release in dissolution medium was observed at 239 nm by UV spectrophotometer.

Nicardipine Hydrochloride Solid – SMEDDs: Liquid solid compact method is used for solid SMEDDS; for the sake of maintaining suitable flow and compression attributes a powder can hold only inadequate concentrations of liquid.

A mathematical approach is used for the determination of concentrations of coating material & carrier materials. This technique is based on liquid retention. The Ø -a value of powder indicates the maximum quantity of non-volatile liquid that can be retained inside its bulk [w/w] along with maintaining an acceptable flowability.

The flowability can be determined from the flow of powder or by measuring the angle of slides [14, 15].

Ø value = Concentration of non-volatile liquid vehicle / Concentration of carrier material

Evaluation of Nicardipine Hydrochloride Solid-Smedds: The Solid-SMEDDS were tested for their micro-meritic properties *i.e*., BD, TD, bangle of repose, % CI & Hausner's ratio.

Differential Scanning Calorimetry: About 5 mg sample was heated in a close pierce aluminium pan at 30 - 80 °C. The rate of heating was 5 °C/minute under a nitrogen stream. The flow rate was kept at 50 ml/min. The samples were prepared and analyzed by differential scanning calorimeter.

Scanning Electron Microscopy: About 1 gm of the Nicardipine Hydrochloride Solid-SMEDDS sample mounted on the stub and then further coated with gold particles and observed in an SEM at an accelerated voltage of 100 kV [6, 10].

Transmission electron microscopy: Transmission electron microscopy samples were prepared by diluting with water. A drop of it was placed over 300 # carbon coated copper grid (Ted pella) and left for 5 min for settling of the droplets. An extra amount of liquid was discarded by filter paper, and the grid was left for air drying. A drop of 1% phosphotungstic acid was added to the grid and kept for 5 minutes for settling and drying as done previously. At last dried grid was observed under Jeol TEM, JEM1010 (Japan) with a voltage operating at 80 kV.

X-ray Powder Diffraction (XRD): The pure drug patterns and optimized solid-SMEDDS formulation patterns were examined by using diffracto meter 5 with a copper target and a scintillation counter detector to know the form of crystallinity and drug crystals intensity in agglomerates [12].

Stability Study: Stability study in accelerated conditions was loaded for the optimized formulation. The S-SMEDDS samples were wrapped in alluminium foil which was laminated and kept in a stability chamber at accelerated condition *i.e.,* at 40 ± 2 °C temperature / 75 ± 5% relative humidity for 03 months. A sampling at predetermined time intervals *i.e.,* 0, 15 & 30 days,

was done. In the testing, different physiochemical parameters were evaluated.

RESULTS AND DISCUSSION:

Solubility Studies: The Solubility of Nicardipine Hydrochloride in different solvents is listed in **Table 3**. The NHCl solubility in various oils decline in sort of Capmul MCM C8 EP > Capmul MCM EP > Captex 300 EP > Labrafil M 1944 CS > Acrysol K-140 > Oleic Acid > Olive oil> Sunflower oil > Castor Oil. The maximum solubility of Nicardipine Hydrochloride was found in Capmul MCM C8 EP *i.e*. 50.73%. Thus for progress of SMEDDS formulation as a oil, Capmul MCM C8 EP was chosen. The solubility of Nicardipine Hydrochloride in various surfactants declines in sort of Labrasol>Kolliphor RH40 > Kolliphor P188 > Tween 80 > Tween 60 > Tween 20.

The highest solubility of the drug was observed in Labrasol, *i.e*. 29.23%. Thus for the progress of SMEDDS formulation as a surfactant, Labrasol was chosen. The solubility of Nicardipine Hydrochloride in various co-surfactants decline in sorting of PEG-400 > PEG-200 > PEG-600 > Capryol PGMC. Thus for the progress of SMEDDS formulation as a co-surfactant PEG 400 *i.e*., 22.98% was chosen.

TABLE 3: SOLUBILITY STUDY OF NICARDIPINE HYDROCHLORIDE IN VARIOUS SOLVENTS

Solvents (OIL)	Solubility (%)	Solvents (Surfactant)	Solubility (%)	Solvents (C0- surfactant)	Solubility (%)
Capmul MCM C8 EP	50.73	Labrasol	29.23	PEG-200	22.98
Capmul MCM EP	30.67	Kolliphor RH40	21.05	PEG-400	30.67
Captex 300 EP	25.76	Kolliphor P188	19.61	PEG-600	18.94
Labrafil M 1944 CS	22.98	Tween 80	14.9	Capryol PGMC	11.92
Acrysol K-140	17.16	Tween 60	18.65		
Oleic Acid	14.13	Tween 20	11.44		
Olive oil	9.95				
Sunflower Oil	8.75				
Castor Oil	5.62				

SMEDDS Characterisation: The % T observed of every batch (NH_1- NH_{13}) was in the range of 99.21% to 100.43% which shows the creation of transparent microemulsion, and it signified that prepared particle size in the structure might be in the range of nanometer. The cloud point of every batch of SMEDDS formulations were observed above 70 °C where the clarity of microemulsion turns into cloudiness which signified that the prepared microemulsion is stable at body temperature [7]. The cloud point of every batch (NH1–NH13) was listed in **Table 4**.

The emulsification time of every batch (NH_1–NH_{13}) was listed in **Table 4**. Due to the low amount of emulsifier surfactant present in the formulation, the emulsification time increased with greater oil concentration and lead to poor emulsification of oil.

The self-micro emulsification efficiency *in-vitro* was examined and was found that it was based on the time of self-micro emulsification, zeta-potential, dispersibility of the droplet size/distribution, & formulation stability. The particle size of all batches (NH_1–NH_{13}) was observed in the range of 92.4 nm to 112.4 nm. The results of particle size showed that particle size enhanced as the concentration of oil enhanced and decrease as the amount of surfactant enhanced. Zeta potential is in the range of -26.40 to -27.40. The globule size & zeta-potential are the key parameters of the colloidal system, which indicates static electricity repulsion and congregation of droplets. As listed in **Table 4**, drug content was observed between 99.00% to 100.40%. Drug content was established within the pharmacopoeial limit.

TABLE 4: RESPONSES OF BOX-BEHNKEN BATCHES

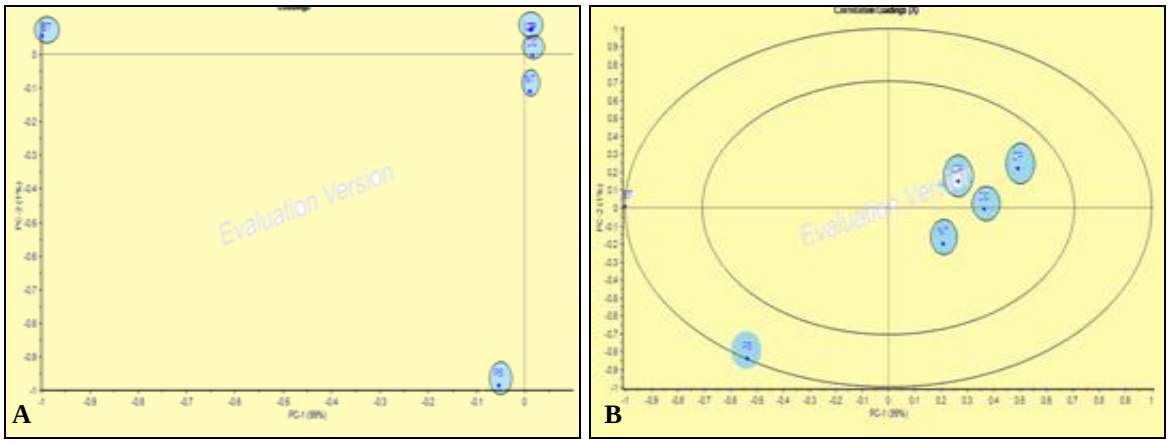
Batch code	Transmission (%)	Emulsification Time (Sec)	Cloud Point (°C)	% Drug Content	Particle Size (nm)	Zeta Potential (mV)
NH1	99.37	38.40	71.67	99.40	102.7	-27.2000
NH2	99.45	41.63	70.33	99.53	108.4	-27.4000
NH3	99.57	19.33	71.33	100.00	92.4	-26.9000

NH4	100.04	25.63	70.67	100.40	98.2	-27.2000
NH5	98.75	35.40	71.00	99.37	100.2	-27.4000
NH6	99.21	42.27	71.00	99.20	109.7	-27.4000
NH7	100.43	20.37	71.33	99.00	92.54	-26.8000
NH8	99.41	27.70	70.67	100.20	100.4	-26.8000
NH9	99.63	56.40	71.33	99.00	112.4	-27.3000
NH10	98.56	43.57	70.33	99.97	107.22	-26.7000
NH11	100.31	49.67	70.00	99.43	106.32	-27.3000
NH12	99.51	28.33	70.67	99.10	94.6	-26.4000
NH13	99.23	32.47	71.00	100.10	98.72	-27.3000

Optimization of Solid SMEDDS: All the responses obtained of all the batches of Nicardipine Hydrochloride SMEDDS like % T, % Release of Drug, Cloud Point (○C), Emulsification time (sec.), % Drug, particle size & zeta potential were examined and listed in **Table 4.** To analyzed the most critical responses which has the most important role in SMEDDS formulation, Principal Component Analysis (PCA) was applied on this set of data using the unscrambler × 10.2 software. The result was received from loading plot **FIG. 2A** it was concluded that 99% of the total variance in the data set was accounted by PC1 (Particle size), and a further 1% was accounted by PC2 (ET).

The outcome of all 13 batches were additionally treated with agglomerative hierarchy cluster analysis (AHCA), and all the formulations were clustered into 5 groups: group I (NH12, NH8, NH4), group II (NH7, NH3) , group III (NH11, NH9), group IV (NH13, NH5) and group V (NH1, NH10, NH6, NH2). The Correlation loading plot (FIG. 2(b)) was constructed to choose the most significant variables for optimization afterward. The results were scrutinized, and Particle size and Emulsification time were found to be the two critical and most important responses on the basis of their eclipse retention. This result showed that particle size and emulsification time are directly proportional to each other. Moreover, all remaining variables were plotted on a correlation loading plot near to origin and thus not discussed. As displayed in 3D plots **FIG. 2C**, the third principal component (*i.e.*, PC3), had no additional variation in the data against PC1 and PC2 and hence is not considered for further studies. In the end, it was fixed that Particle size and emulsification time are the most vital variables in the grounding of SMEDDS loaded with Nicardipine Hydrochloride, and thus, they were further chosen for the study. From the study of PCA, it was observed that from all 13 batches, equally dependent variable Particle size (Y1) and Emulsification time (Y2) exhibits abroad variations from 92.4-112.40 nm and 19.33-49.67 sec, respectively **Table 3**.

A Box–Behnken statistical experimental design with 3 factors and 3 Levels requires twelve experimental runs with one center point, i.e., totally13 batches. The batches were optimized and analyzed, and it was known that the best fit model for it was a non-linear quadratic model, and the comparative R2 values are listed in **Table 4** along with the regression equation generated for both responses. In order for the model, effective the P values must be < 0.05 for a model in order to best fit the quadratic model. The model P values for each dependent variable or response are in **Table 4**.



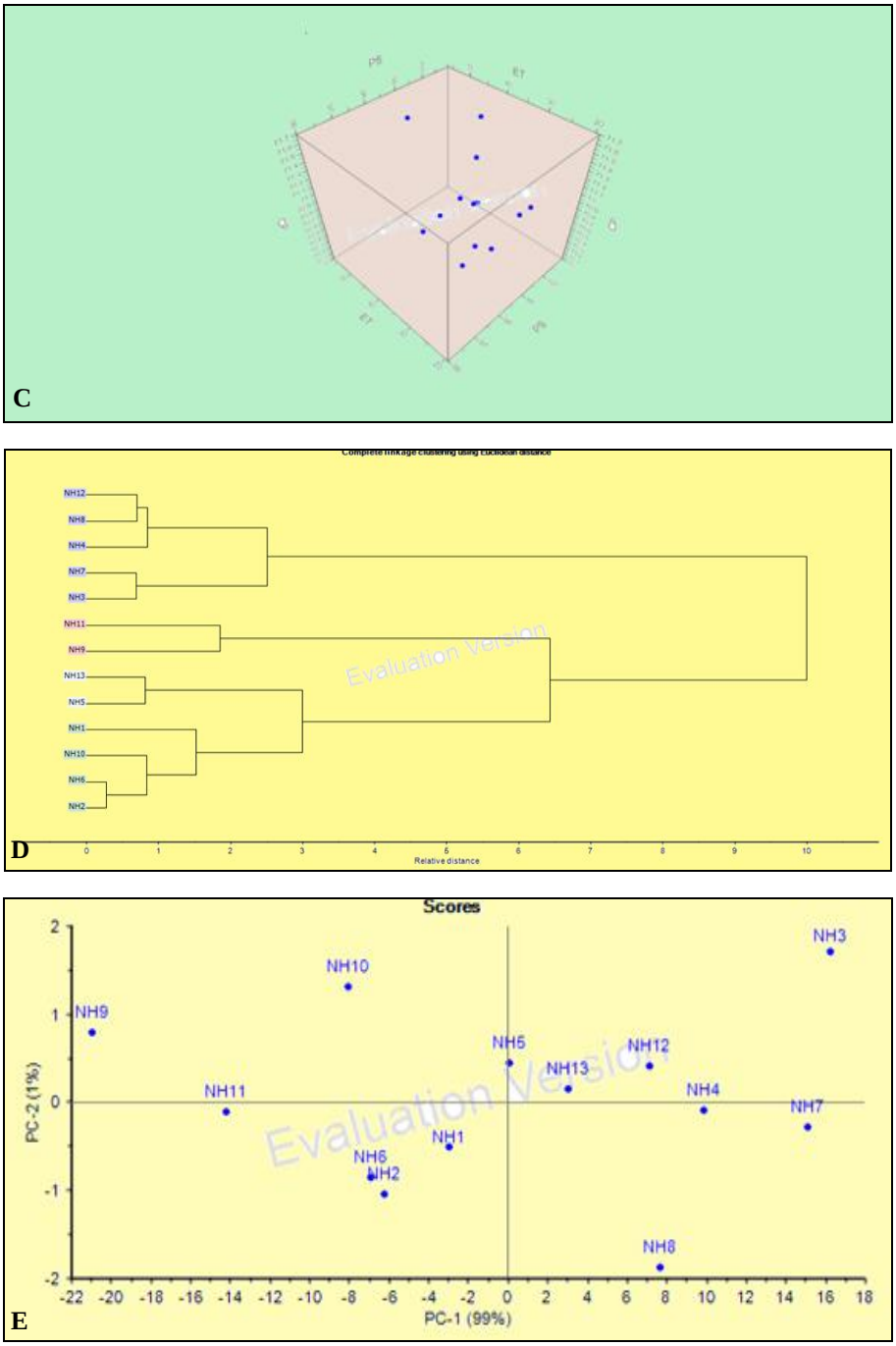


FIG. 2: PCA STUDY FOR NICARDIPINE HYDROCHLORIDE SMEDDS (A) LOADING PLOT(B) CORRELATION LOADING PLOT(C) 3-D SCORE PLOT(D) DENDOGRAM(E) SCORES PLOT

Formulation Composition Factors Influence On Responses: From the ANOVA test, it was observed that as the model P values are < than 0.05 values for both Y1 and Y2 responses *i.e.* particle size and emulsification time and thus responses (Y1 & Y2) fitted best with polynomial quadratic model. Also, R2 values of both responses Y1 & Y2 are 0.9933 & 0.9879 respectively. Emulsification time = 32.47 + 2.97 X1 – 8.65 X2 – 6.45 X3 + 0.766 X1 X2+ + 0.116 X1 X3 – 2.13 X2 X3 – 7.14 X12 + 5.92 X22 + 6.10 X32 Equations (1)**.** The practical value of Emulsification time for all the batches ranged from 19.33 sec to 56.40 sec. The result influenced the three independent variables, X1 (2.97), X2 (-8.65) and X3 (-6.45) and the independent variables on Y1 shows the main effect in disturbing Y1. The X1 ($p < 0.05$) was established to be major factor that affects Y1. X1 has positive sign of co-efficient, screens positive effect on emulsification time. The X2 (-8.65) and X3 (-6.45) with P value < than 0.05 was established to be significant factor that affects Y1. The X2 and

X3 has negative sign of co-efficient; showing antagonist effect on Y1 which means it decreases emulsification time. Particle size = 98.72 + 3.61 X1 – 4.68 X2 – 4.46 X3 + 0.025 X1 X2+ -0.410 X1 X3 – 1.63 X2 X3 – 1.36 X12 + 3.07 X22 + 3.35 X32 Equations (2)

The practical value of Particle size of all the batches ranges from 92.4-112.4 nm. The statistics indicated that independent variables selected for the study strongly influenced Y2.

The 3 independent variables, the X1 (3.61), X2 (-4.68), and X3 (-4.46) showed the main effect in disturbing Y2. The X1 ($p < 0.05$) was known to be significant in affecting Y2. X1 has a positive sign of co-efficient, showing a positive effect in increasing the particle size. The X2 (-4.68) and X3 (-4.46) with P values < than 0.05 was found to be most significant factor in affecting Y2.

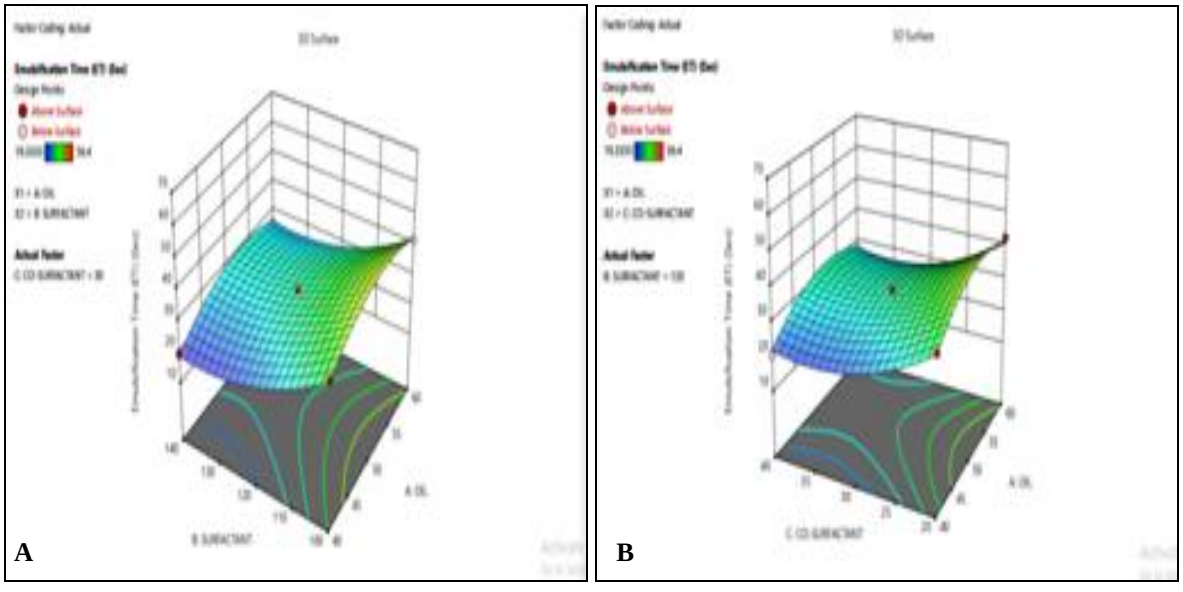
The X2 and X3 have a negative sign of co-efficient; showing antagonist effect on Y2 means decreasing the size of the particle.

The plots were generated *i.e.*, 2D contour plots and 3D response surface plots representing interaction effects of the factors on the variables are shown in **Fig. 4**. The correlation between the independent and dependent variables was extra enlightened by the use of the response surface plot. **Fig. 4A** which shows an interaction effect between oil (Capmul MCM C8 EP) and surfactant (Labrasol) on the emulsification time as dependent variable.

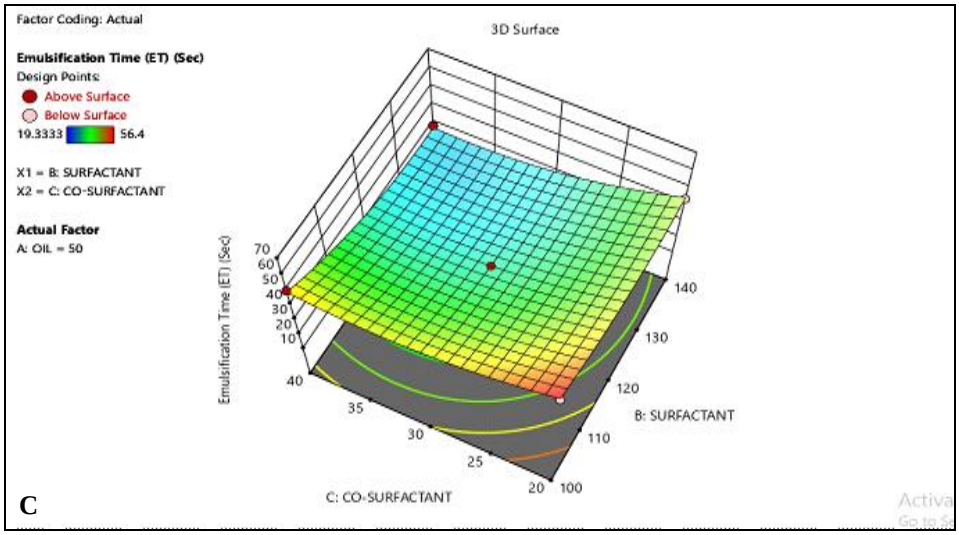
The inverse relationship between concen-tration of oil to the particle size and emulsification time indicates a linear decline of particle size and emulsification time with a decrease in the amount of oil (Capmul MCM C8 EP) and increases in the amount of surfactant (Labrasol).

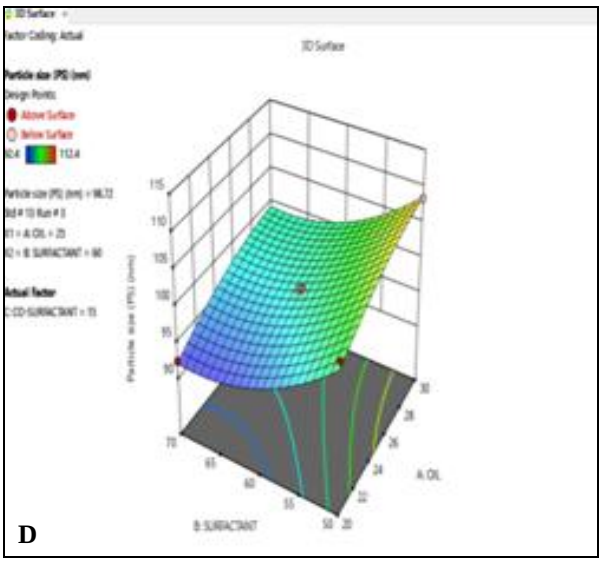
TABLE 5: DEPENDENT VARIABLES

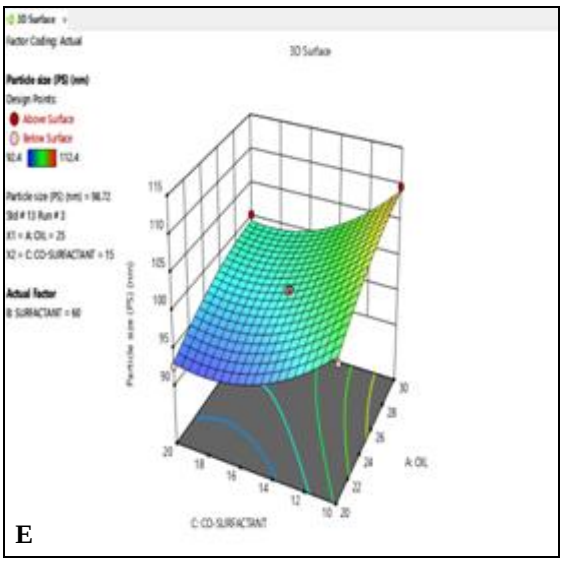
Dependent variables	Emulsification time (Y1)		Particle size (Y2)	
	Coefficient	P value	Coefficient	P value
Intercept	32.47	0.0042	98.72	0.01
X1	2.97	0.0196	3.61	0.0059
X2	-8.65	0.0009	-4.68	0.0028
X3	-6.45	0.0022	-4.46	0.0032
X1X2	0.766	0.4648	0.025	0.9746
X1X3	0.116	0.9069	-0.41	0.6108
X2X3	-2.13	0.1035	-1.63	0.1091
X12	-7.14	0.0098	-1.36	0.2507
X22	5.92	0.0165	3.07	0.6493
X32	6.1	0.0152	3.35	0.0396
	F value= 49.25 R2 value= 0.9933 Model P value < 0.0042		F value= 27.32 R2 value= 0.9879 Model P value < 0.0100	

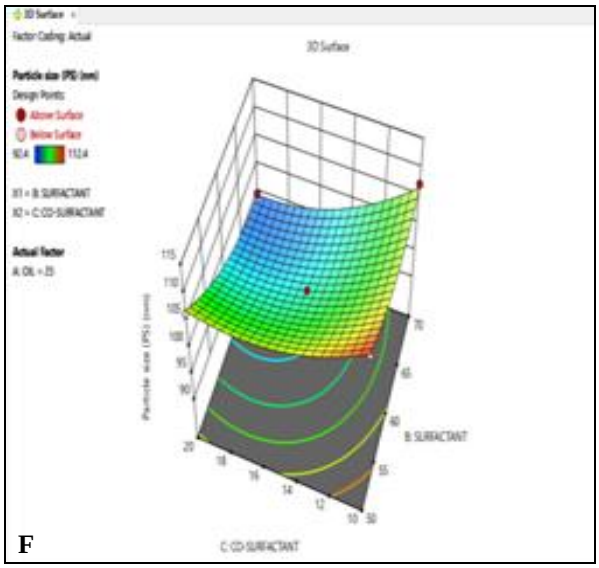


A **B**









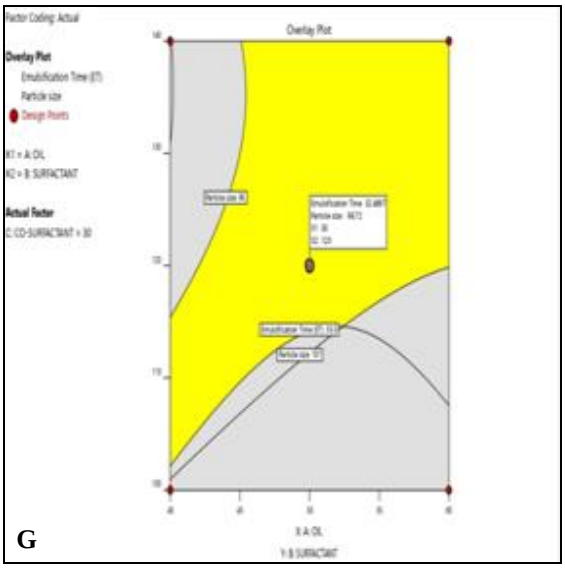


FIG. 3A: 3D SURFACE PLOTS SHOWING EFFECT OF X1 AND X2 ON Y1 B)3D SURFACE PLOTS SHOWING EFFECT OF X1 AND X3 ON Y1 C) 3D SURFACE PLOTS SHOWING EFFECT OF X2 AND X3 ON Y1 D) 3D SURFACE PLOTS SHOWING EFFECT OF X1 AND X2 ON Y2 E) 3D SURFACE PLOTS SHOWING EFFECT OF X1 AND X3 ON Y2 F)3D SURFACE PLOTS SHOWING EFFECT OF X2 AND X3 ON Y2G)OVERLAY PLOT OF OPTIMIZED BATCH

Optimized formulation validation: By using software optimization and response surface plots, levels elected were X1, X2, and X3 for 50, 120, and 30 mg, respectively. Additionally, this value was fitted in Equations (1) & (2) which gives the theoretical value of 32.4667 seconds for emulsification time and 98.72 nm for particle size, respectively. Newly formulation was marked ready by using the independent variables optimum levels. The observed values of Particle size and emulsification time were found to be 98.40 and 32.73 ± 1.72 sec, respectively, which were in close relation with the theoretical values.

Characterization Nicardipine SMEDDS: Characterization of SMEDDS below droplet size 300 nm was performed, and the globule size and PDI were observed as 97.745 nm and 0.227, respectively. A PDI value below 0.3 is indicative of good uniformity in the distribution of the size of the globule after dilution with water. One of the crucial factors for SMEDDS is droplet size, as it follows the extent and rate of drug release as well as its absorption. Moreover, it has been reported that a globule of smaller size in microemulsion leads to a rapid absorption to improve its dissolution rate. It was observed that the surfactant concentration & co-surfactant concentration had an inverse relationship; the concentration of surfactant was directly proportional to oil concentration.

The type of co-surfactant was not considerably affecting the size of the droplet, but the surfactant (propylene glycol) containing the microemulsion were producing large droplets size with a higher viscosity. The increase in the oil phase (castor oil) ratio resulted in a proportional increase in particle size. It's known that surfactant addition to these systems causes interfacial tension to condense and make it steady, whereas co-surfactant addition results in film expansion, and thus a proportion relative of surfactant to co-surfactant was found to have a variable effect on the size of the globule. Moreover, it's been reported that the small size of particles of the emulsion globule is leading to a rapid absorption & bioavailability improvement.

TABLE 6: EVALUATION PARAMETER OF NICARDIPINE SMEDDS

Parameter	Value
Globule size	97.745 nm
PDI	0.227
Zeta potential	-27.5 mv
% Transmittance	99.50 ± 0.26 %
Emulsification time	32.73 ± 1.72 sec
Cloud point	71.47 ± 0.21 °C

Zeta-Potential of Optimized Formulation: In general, with gaining of repulsive forces electrostatic in nature between microemulsion globules, a coalescence of droplets of microemulsion is inhibited. In divergence, with a decreasing electrostatic repulsive force, separation of phase occurs.

An anisotropic mixture of oil, surfactant, and co-surfactant is called SMEDDS 3. Formulation of globules in SMEDDS occurs only when it is diluted in water media. So during the storage of SMEDDS, there is no formation of aggregates of globules.

A value of -27.5 mV for the Zeta-potential of SMEDDS was found. A more negative value than 25 mV of zeta potential are considered as stable normally. A value of -28.5 mV for zeta potential of S-SMEDDS was found.

Robustness after Dilution of Optimised Formulation: % Transmittance was found to increase as dilution was increased. By dilution of SMEDDS to 50 times, 100 times, 500 times & 1000 times with 0.1 N HCL, no sign of phase separation or drug precipitation in optimized formulation after 24 hrs was observed. The obtained results revealed that optimized Nicardipine Hydrochloride SMEDDS exhibited excellent stability with no sign of phase separation or drug precipitation at stress condition variability, *i.e.*, cooling-heating cycle (at 2-8, 45 and 60 ºC), centrifugation test at 1000 rpm after 48 hrs.

***In-vitro* Drug Release Study of Optimized Formulation:** *In-vitro* drug release study of optimized formulation was shown in **FIG. 4,** and % drug release was found to 90.37% at 15 min.

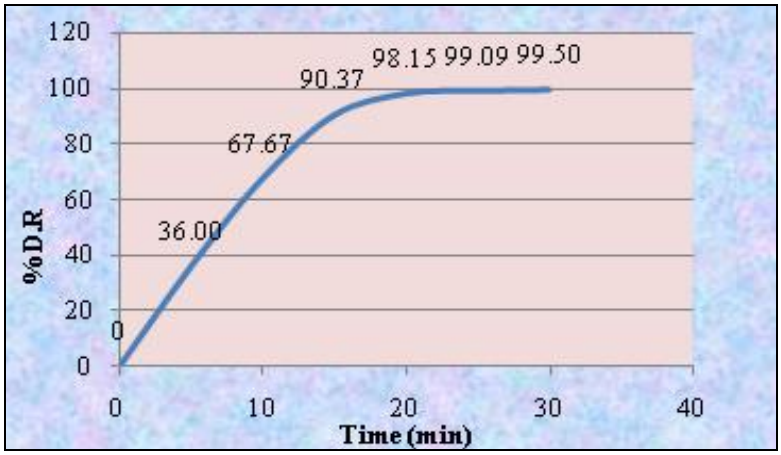


FIG. 4: DRUG RELEASE STUDY OF OPTIMIZED BATCH

S-SMEDDS Evaluation:

Angle of Slide Determination: The angle of slide measurement was performed of three carriers as Neusilin US2, Fujicalin, and MCC 102, and on the basis of Ø Value, the Neusilin US2 (0.74) was selected as carrier material.

FIG. 5: MEASUREMENT OF ANGLE OF SLIDE

It was revealed that solid adsorbents such as Fujicalin and MCC 102 did not have good adsorption capacity in comparison to Neusilin US2. So, Neusilin US2 was further selected for the study. The free-flowing S-SMEDDS was prepared. By optimized formulation of liquid SMEDDS.

Determination of Liquid Load Factors (Lf):

Loading Factor Was Calculated by Equation:

Lf= Øcarrier + Øcoating (1/R)

Lf= 0.74+3.26 (1/20)

Lf= 0.72 + 0.163

Lf= W/Q

Q = W/ Lf

Q = 200/0.903

R = Q/q

q = Q/R

q = 221.48/20

TABLE 7: FORMULATION OF NICARDIPINE HYDROCHLORIDE SOLID SMEDDS

Ingredient	Use	Quantity (mg) (For one Tablet)
Nicardipine HCl SMEDDS	Liquid medicament	200
Neusilin US2	Carrier	221.48
Aerosil 200	Coating agent	11.07
Crosscarmellose sodium	Super disintegrant	15
Microcrystalline cellulose	Diluent	42.45
Magnesium stearate	Lubricant	10
Total		500

TABLE 8: EVALUATION PARAMETER OF SOLID SMEDDS

Evaluation of S-SMEDDS	
Specification	**Value**
Globule size	98.165 nm
PDI	0.236
Zeta potential	-28.5
% Transmittance	99.40 ± 0.2 %
Cloud point	72.70 ± 0.79°C
Emulsification time	33.13 ± 1.22 sec
Drug content (%)	99.45 ± 0.14

TABLE 9: FLOW CHARACTERISTIC OF SOLID SMEDDS

Characteristic	Result
Angle of repose	24.10° ± 0.140°
Bulk density	0.317 ± 0.004 gm/ml
Tapped density	0.370 ± 0.014 gm/ml
Carr's index	14.21 ± 4.12 %
Hausner's ratio	1.16 ± 0.060

Differential Scanning Calorimetry of Optimised S-SMEDDS Formulation: As shown in **Fig. 6A**. Thermal behavior of the pure drug Nicardipine Hydrochloride and **6B** thermal behavior of the solid-SMEDDS of Nicardipine Hydrochloride. Pure Nicardipine Hydrochloride shows a characteristic sharp endothermic peak at 171.28 °C. A sharp peak is the primary indication of the crystalline nature of pure drug. Now, DSC of solid SMEDDS showed no peak at 171.8 °C it indicates that the drug was completely converted into amorphous form or was present in solubilized form.

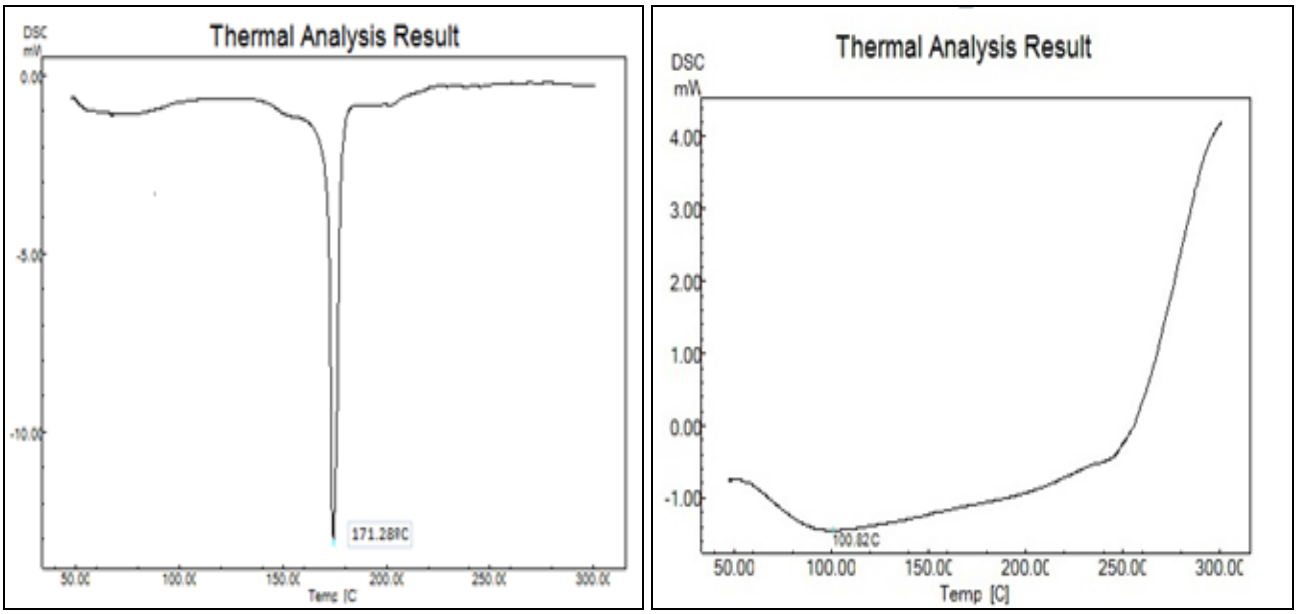


FIG. 6: DSC THERMOGRAMS (A) PURE NICARDIPINE HYDROCHLORIDE (B) S- SMEDDS

Powder X-ray Diffraction of S-SMEDDS Optimised Formulation: Physical state of the drug in the Solid SMEDDS was verified by PXRD. In **Fig. 7** presence of sharp peaks indicates the presence of Nicardipine Hydrochloride in a highly crystalline form.

As it can be seen from the absence of sharp diffraction patterns, Neusilin US2 is in amorphous state. The physical mixture (1:1) of Nicardipine Hydrochloride and Neusilin US2 were showing some crystalline peaks due to the presence of Nicardipine Hydrochloride in the mixture.

In contrast to the physical mixture of Nicardipine Hydrochloride and Neusilin US2, the Solid SMEDDS did not show significant crystalline peaks, which further confirmed the molecularly dispersion state of Nicardipine Hydrochloride in the proposed formulation

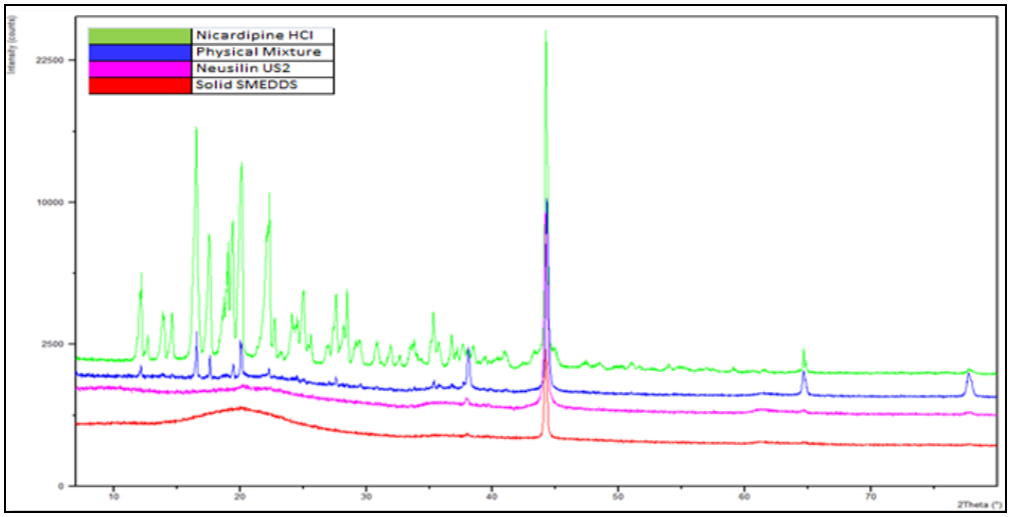


FIG. 7: POWDER X-RAY DIFFRACTION (PXRD) OF NICARDIPINE HYCHLORIDE, PHYSICAL MIXTURE (NICARDIPINE HYCHLORIDE AND NEUSILIN US2), NEUSILIN US2, AND SOLID SMEDDS

Scanning Electron Microscopy: The scanning electron microscopy of optimized S-SMEDDS formulation Nicardipine Hydrochloride (pure API), Neusilin US2 (carrier material) & S-SMEDDS are shown in **Fig. 8**A Pure drug powder appears to be crystalline & **8B** irregular shaped. The Neusilin US2 appeared with a rough surface with pores. However, **8C** S-SMEDDS appeared as smooth-surfaced, which indicated that the liquid SMEDDS is adsorbed or coated within the pores of Neusilin US2.

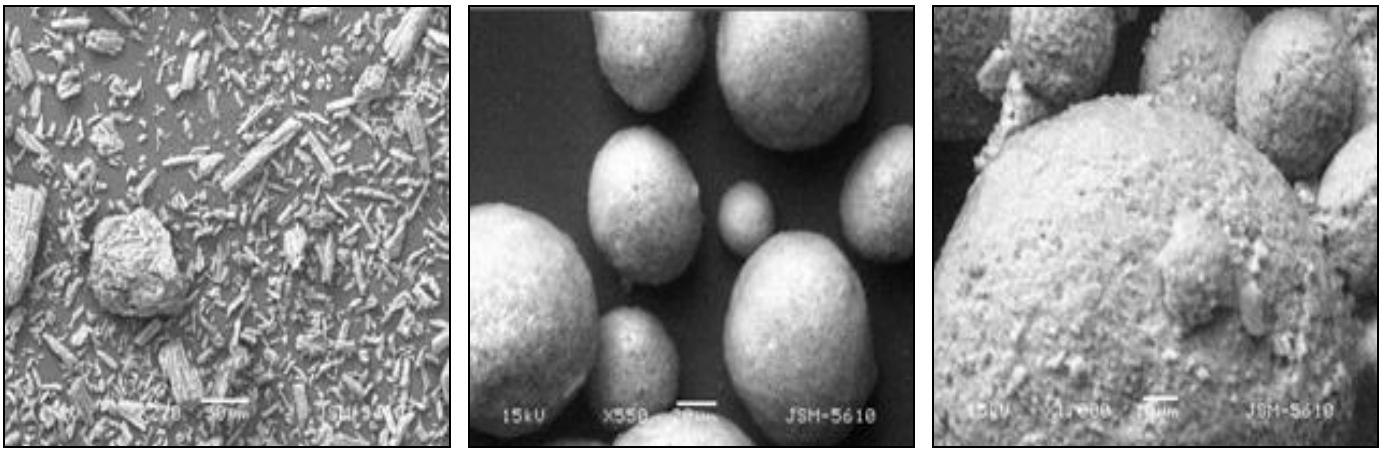


FIG. 8: SEM OF (A) NICARDIPINE HYDROCHLORIDE (B) NEUSILIN US2 (C) S-SMEDDS

Transmission Electron Microscopy (TEM): TEM image subsequent to post-dilution of NHCl-loaded Solid SMEDDS with distilled water is shown in Fig. 9 and is interpreted for globule size & surface morphology. The figures shown showed that globules of all composition formulas were well dispersed, and no aggregation of globule was observed.

It was revealed by TEM analysis that most formulas showed homogenous spherical droplets, which satisfies the criteria of Micro emulsifying formulae.

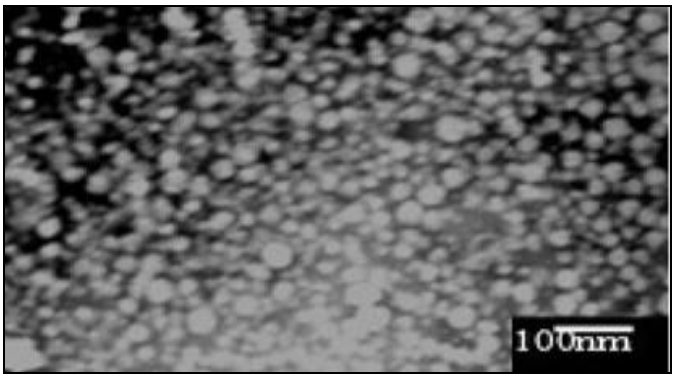


FIG. 9: TEM PHTONMICROGRAPH FOR THE OPTIMIZED BATCHES NH-LOADED SOLID SMEDDS

***In-vitro* Drug Release Study of Nicardipine Hydrochloride S-SMEDDS:** *In-vitro* drug release study of S-SMEDDS of Nicardipine Hydrochloride was shown in **Fig. 10.** The result found that Nicardipine Hydrochloride loaded solid SMEDDS shows drug release of 90.82% at 15 min.

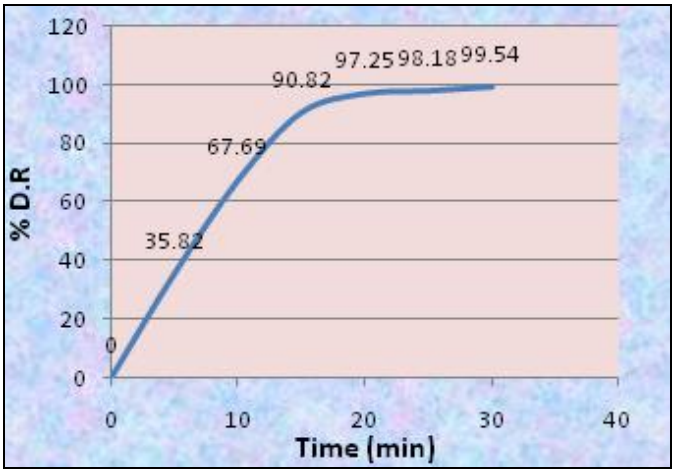


FIG. 10: DRUG RELEASE STUDY OF S-SMEDDS

Accelerated Stability Studies: Accelerated stability study of the optimized formulation revealed no variable changes in the physical parameters when they were stored at 40 ± 2 °C/75 ± 5% RH room temperature respectively for 3 months. The characteristic peaks of Nicardipine Hydrochloride present in FTIR spectra showed no degradation of the drug. So, in the conclusion of the stability, the formulation which was optimized of Nicardipine Hydrochloride S-SMEDDS was stable.

CONCLUSION: S-SMEDDS were prepared by a novel technique to deliver poor water-soluble drugs with an increased dissolution rate. The drug candidate Nicardipine Hydrochloride has limited solubility. SMEDDS of the drug were prepared successfully by using Labrasol surfactant and PEG-400 co-Surfactant, and Capmul MCM C8 EP oil.

The liquid-formulation which was optimized consist Nicardipine Hydrochloride (20 mg), Labrasol (120 mg), PEG-400 (30 mg), Capmul MCM C8 EP (50 mg). The % T of the formulation which was optimized was 99.50 ± 0.26%, and desired formation of microemulsion with a size of globule is < 100 nm. An emulsification time of 32.73 ± 1.72 seconds was observed. A Cloud point of 71.47 ± 0.21 ºC suggested stability of emulsion formation at physiological temperature.

The zeta potential and size of globule of the formulation which as optimized was found to be 28.1 mV and 152.1 nm, respectively. The *in-vitro* drug release study for the formulation was found to be 86.57 ± 0.65 at 30 min. The superiority of Nicardipine Hydrochloride S-SMEDDS over the commercial formulation w.r.t *in-vitro* dissolution profile was observed. Thus, considering S-SMEDDS as a novel and commercially feasible formulation of Nicardipine Hydrochloride with intensified properties for dissolution.

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DISCLOSURE STATEMENT: In accordance with the journal policy and my own ethical obligation of being a researcher, I report that Rajesh L Dumpala and Dr. Akruti Khodakiya are the named researchers of this work and No conflict of interest is reported by the authors for this work and they are responsible for paper writing and content.

CONFLICTS OF INTEREST: The authors confirm no conflict of interest for this manuscript.

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